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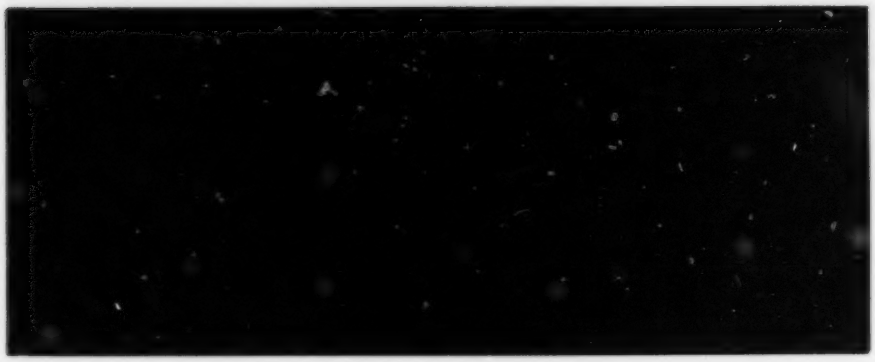

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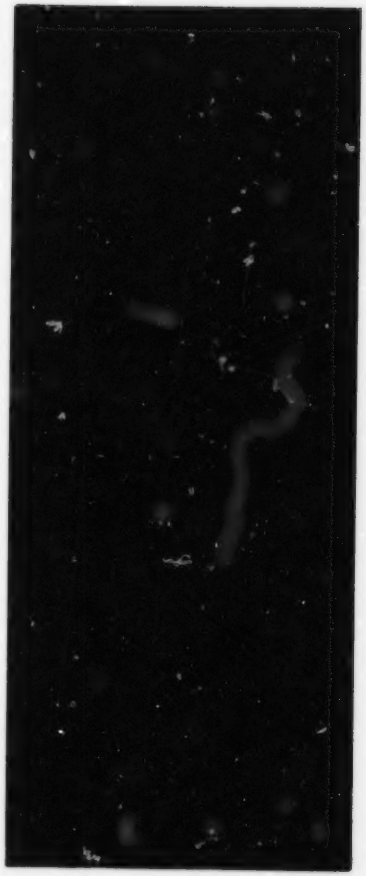

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FINAL REPORT

# A STUDY OF VITAMIN A TOXICOSIS IN GROWING SWINE

Project No. 2.4-37

Saskatchewan Agricultural Development Fund

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## Executive Summary


### Abstract

Objectives of this research were to experimentally reproduce vitamin A toxicosis, to study the disease that developed and to compare experimental findings to those seen in certain naturally occurring skeletal diseases in Saskatchewan.

Rations high in vitamin A caused marked lameness and increased serum and liver levels of retinyl palmitate.. Premature growth plate closure occurred in many long bones but not in vertebrae. There was no evidence of narrowing of the vertebra canal. These results provide new information about vitamin A toxicity in livestock but suggest that factors other than excess vitamin A may be operative in naturally occurring cases in bone disease.


### Summary

A syndrome of stunted growth, long bone deformity and paralysis has been seen in pigs and calves in Saskatchewan and in other parts of western Canada. Outbreaks of this disease have been investigated but the cause has not been determined. The basic abnormality in affected animals appears to be premature closure of growth plates - especially in long bones and in vertebrae. If vertebrae are involved, there is narrowing of the spinal canal, pressure on the spinal cord and paralysis. Affected animals do not survive.

Vitamin A toxicosis (excess vitamin A in the feed or given by injection) is one of the possible causes of the above disease. While it is known that high doses vitamin A will cause premature closure of growth plates, it is not known if the spine is affected nor are the details of the pathological changes that occur in various tissues of the body well documented. With the above information in mind, we undertook to produce vitamin A toxicosis in swine. It

was our intention to study the clinical signs and postmortem findings with special reference to the skeleton and to compare these changes to the naturally occurring disease that is described above.

Weanling pigs (5 per group) were fed high doses of vitamin A (500 times NRC recommended levels) for periods of 7 and 27 days. Control animals were fed similar rations that contained vitamin A at NRC recommended levels.

Beginning at 7 to 17 days pigs fed high levels of vitamin A developed stiffness and were reluctant to rise. Fetlocks were over extended, pigs stood with their back arched and towards the end of the trial, treated pigs continued to eat but spent most of their time lying on their side. Performance was not affected by high vitamin A diets although we have the impression that weight gain and feed consumption would have been reduced if the experiment had continued for a longer period of time. Treated pigs had a mild degree of anemia (at day 27) when compared to control animals.

Levels of vitamin A were measured in blood serum and in liver at the beginning of the experiment and at the end of a 7 and 27 day feeding period. Levels of vitamin A were elevated in pigs fed high vitamin A rations at both 7 and 27 days. Liver levels of vitamin A were much higher (approximately 90 times the amount stored in the liver of control animals) at the end of the 27 day period. There was considerable variation in serum and liver vitamin A levels in pigs fed high levels of vitamin A and animals with the highest levels of vitamin A did not necessarily have the most severe clinical signs. It became apparent that vitamin A, when stored in excess in the liver, is largely in the form retinyl palmitate.

At postmortem examination 1 pig in the 7 day group and 4 of the 5 pigs in the 27 day group (high vitamin A groups) had narrowing and premature closure of growth plates of long bones of the limbs. This was not present in any of the control animals. Vertebrae were not affected and there was no deformity of any part of the skeleton. Vitamin A storing cells (Ito cells) were identified microscopically in liver and in adrenal glands. The number and size of these cells increased, over time, in pigs fed excess vitamin A and, in a naturally occurring case of vitamin A toxicity, would provide a clue to the investigator.

While this study fails to clearly implicate excess vitamin A as the cause of naturally occurring skeletal disease in Saskatchewan livestock, the results of this research do provide us with the better understanding of the harmful affects of this vitamin if fed in excessive amounts. Data relating to clinical signs, postmortem findings as well as the blood and liver levels of vitamin A have direct application in veterinary diagnostic laboratories and to practicing veterinarians. Findings in regard to growth plates of the skeleton are of particular interest since many bone diseases in livestock are poorly understood and often go undiagnosed in terms of establishing the cause.

Finally, a report of this nature should remind livestock producers that nutrients such as vitamin A are potent in small amounts and are potentially very harmful if fed in excess amounts.

## A Study of Vitamin A Toxicosis in Growing Pigs

### Background and Objectives

A syndrome of stunted growth, bone deformity and paralysis occurs sporadically in pigs and calves in Saskatchewan and in other parts of western Canada. Apparent outbreaks of this type of disease have been investigated on a number of Saskatchewan farms but the cause has not been established. The basic abnormality appears to be premature closure of growth plates in various parts of the skeleton. If vertebrae are involved, there is narrowing of the vertebral canal, pressure on the spinal cord and paralysis. Affected animals may survive for several weeks or months but all eventually die.

Since vitamin A toxicosis is one of the possible causes of this skeletal disease, the experimental reproduction of vitamin A toxicosis was undertaken in an attempt to study and compare the above experimental lesions to the naturally occurring disease with a view to establishing the cause of the latter.

Specific objectives were a) to produce vitamin A toxicosis in growing piglets, b) to document clinical signs and postmortem findings with special reference to growth plates of the skeleton and c) to compare the experimental and naturally occurring lesions.

### Experimental Methods

Twenty-five recently weaned York-Landrace cross piglets formed the basis of this experiment. Animals were obtained from a single source, minimal disease herd at a mean weight of 8.6 kg and mean age of 32 days. At 28 days of age, the piglets had been weaned onto a commercial creep feed. Upon purchase, animals were transported to the Western College of Veterinary Medicine Animal Care Unit where they were fed the control diet and allowed to acclimatize for three days prior to the beginning of the experiment. Piglets were randomly assigned to one of the five tests groups; each group contained five animals - three castrate males, and two females. Piglets were housed in individual pens (2.5 $m^2$) on a wire mesh floor with individual feeders and waterers. All animals were observed twice daily and any clinical signs were recorded. Individual body weights of all animals were obtained weekly; photographs were taken where appropriate. All animals were fed ad libitum twice daily except

for group 4 whose intake was adjusted to match the intake of pigs in group 5. Feed consumption was calculated on a daily basis. The control diet and the high vitamin A diet were similar and were formulated as wheat based starter meal rations; high vitamin A rations differed only in that they contained additional vitamin A and chromic oxide as a marker. Rations were analyzed for protein, calcium, phosphorous and digestible energy soon after formulation; values obtained met or slightly exceeded NRC (1988) requirements for 10 - 20 kg piglets. Vitamin A analysis of the feed was carried out approximately three months after formulation using high performance liquid chromatography[a]. The results of these assays are given in Table 1. Details of the experimental rations and of the experimental design are given in Tables 1-4.

Blood samples were collected from the anterior vena cava of all pigs on day 0 and at the time of slaughter (day 7 or day 27). Harvested serum was frozen, stored at -20°C and assayed for retinyl palmitate and retinol by high performance liquid chromatography[b]. Hematologic and serum chemistry values (complete blood count, Na, K, Cl, $Co_2$, Ca, P, Mg, urea, creatinine, glucose, total bilirubin, alkaline phosphatase, total protein, aspartate aminotransferase, creatinine phosphokinase, gamma glutamyl transferase, total protein and albumin) were also determined from the blood samples obtained.

Piglets were euthanized by the intravenous administration of an overdose of barbiturate**[c]. Pigs were then immediately bled-out through the femoral artery and necropsied. All body systems were examined and fresh liver weights were recorded and expressed as a percentage of total body weight. Samples of liver and distal femoral growth plate cartilage were fixed in 5% gluteraldehyde. Tissue samples of liver, kidney, lung, trachea, parathyroid gland, thyroid gland, adrenal gland, spleen, thymus, inguinal lymph node, skin and stifle synovium were fixed in 10% neutral buffered formalin. In addition to the above samples, longitudinal mid-sagittal bone sections were obtained from the following sites; sixth rib, femur, tibia, rear limb phalanges, humerus, sixth thoracic vertebra, frontal bone, tuber ischium, radius, ulna, metacarpus, and cranial base. These tissues were also fixed in 10% neutral buffered formalin. Growth plates at all of the above sites were examined under a magnifying glass and free hand drawings were made. Photographs and x-rays

a) Hoffman La Roche, Etobicoke, Ontario
b) Animal Health Diagnostic Laboratory, Lansing, Michigan
c) Euthanyl Forte, MTC, Cambridge, Ontario

were taken of slab sections of the left femur, left humerus and sixth thoracic vertebra. The length of the right femur and humerus was measured at the greatest perpendicular distance between the proximal and distal articular surfaces. The length and width of the sixth thoracic vertebra was determined from mid-sagittal sections of this bone. These measurements were then expressed as a percentage of total body weight. All formalized tissues were trimmed, embedded in paraffin, sectioned at 6 $\mu$ and stained with hematoxylin-eosin. Bone sections were decalcified in 20% formic acid. Selected tissues were stained with saffarin-o fast-green; frozen sections of liver were stained with oil-red-o. The second last thoracic vertebra was isolated at necropsy and frozen at -20°C. At a later date this vertebra was boiled to removed soft tissue and the width and height of the vertebral canal was measured using calipers.

A portion of each liver was obtained at necropsy and stored (by freezing at -20°C) and later assayed (on a dry weight basis) for retinyl palmitate and retinol by high performance chromatography[d].

All histologic slides were first examined with the knowledge of the group number; subsequently all tissues were re-examined blindly and lesions described and recorded. The H & E stained hepatic sections were evaluated for the number of Ito cells by counting the number of perisinusoidal cells bearing large lipid vacuoles in one oil immersion field (1000x magnification) in the periportal zone of 5 lobules. Oil red-o stained hepatic sections were evaluated for the number and distribution of Ito cells by subjectively assessing the number of perisinusoidal cells bearing large red vacuoles in the periportal, mid zonal and central lobular area of the lobules in the entire section and the assigning a score of 0 to 3. H & E stained sections of adrenal glands were evaluated for the number of perisinusoidal cells bearing large lipid vacuoles by counting cells in 5 oil immersion fields (1000x) in the zona fasciculata adjacent to the zona glomerulosa and by assessing the vacuolation of epithelial cells.

All interval continuous data (body and organ weight, hematology, chemistry, Vitamin A levels, bone measurements, and feed intake) were analyzed using a one-way analysis of variance. Differences amongst means were determined using a Student-Newman-Keuls multiple range test. Differences were assumed to exist when the probability of making a Type I error was less than 5%

d) Animal Health Diagnostic Laboratory, Lansing, Michigan

## Results

### Clinical Signs, Performance and Clinical Pathology

Control pigs remained clinically normal throughout the course of the experiment; all control pigs remain bright and alert, appetite was good and there was no evidence of lameness. In contrast, many of the pigs fed the high vitamin A ration (treatment group) developed some locomotory abnormality. The severity and time of onset of lameness was somewhat variable; the earliest change observed in treated pigs was a mild lameness that occurred around day 7. However, 2 pigs in the 7 day trial had no signs at this time and 2 of the 5 piglets in the 27 day trial did not develop abnormalities in locomotion until day 13 and day 17.

The lameness that occurred in treated pigs was characterized by stiffness, a slight shortening in stride, and moderate reluctance to rise. The affected animals stood with their feet placed well under the body and with the back slightly arched; treading with the hind legs was commonly observed. Fetlocks (usually first noticed in their rear limbs) were slightly over extended and some pigs tended to assume a dog-sitting position. When increased activity occurred, as in the movement of pigs for photography, treated pigs appeared to tire more easily than control pigs and lameness was often worse the next day. In the 27 day trial, the clinical signs worsened with the days on feed. By day 27, pigs #43, 51 and 56 spent most of their time in lateral recumbency, had considerable difficulty in rising and walked with hesitation. Fetlocks of these animals had marked over extension and, in weight bearing, almost touched the ground. The most severely affected pigs in the treatment group (#43, 51, 56) developed a chronic dermatitis around their ear tags which had been inserted on day 0. This dermatitis was characterized by the presence of small amounts of purulent exudate, much reddish brown crusting, and by a tendency to bleed when traumatized. In addition, these pigs had a dry scruffy hair coat (generalized) and 2 animals (#43 and 56) had reddening of the oral mucocutaneous junctions. Except for pigs #43, 51 and 56 who appeared to be mildly depressed towards the end of the experiment, all pigs in the treatment group remained bright and alert. Treated pigs, in general, had a good appetite

throughout the experiment. The most severely affected animals had some difficulty getting to their feed trough. However, once at the trough, they ate well, usually while kneeling or while in sternal recumbency.

No significant differences in feed intake or weight gain were detected. Of the clinical pathology data, only the PCV (packed red cell volume) and total serum protein values were affected by high vitamin A rations. Pigs fed high vitamin A rations for 27 days (group 5) had a significant elevation in their total serum protein and their packed red cell volume was reduced.

**Vitamin A Levels in Liver and Serum**

On day 0 all experimental animals had similar levels of vitamin A in their serum (tables 5 & 6). At the end of the day 7 experiment, treated animals (group 2) had significantly elevated levels of total vitamin A in their serum and liver. The increase in serum vitamin A was due to an increase in the retinyl palmitate values; levels of serum retinol did not change. On day 7 hepatic levels of total vitamin A in group 2 animals were increased (approximately 19x) due to a significant increase in both retinol and retinyl palmitate.

On day 27 total serum vitamin A was elevated (approximately 4x control levels) in group 5 animals; values were comparable to those seen at day 7. Again, levels of serum retinol were not affected by the high vitamin A diet. At this point (27 days) total vitamin A stored in the livers of pigs in group 5 was increased approximately 90 times over that stored in the livers of control animals. While liver retinol showed a moderate increase, most of the vitamin A stored in the liver was in the form of retinyl palmitate. There was marked individual variation in the levels of retinyl palmitate in the serum of pigs in group 5 (table 5). Animals with the most severe lesions did not necessarily have the highest levels of vitamin A in serum or liver.

**Gross Postmortem Findings**

Although gross findings were largely confined to the skeleton, a few lesions were observed in soft tissues. Two pigs in group 5 (number's 39 & 41) had livers that were yellow-brown in color. There were no significant

differences in liver weights between the control and the treatment groups. Lesions where ear tags had been inserted and changes involving oral mucocutaneous junctions are described in the section on clinical signs.

Gross lesions involving the skeleton were detected in three pigs - all in group 5. In no case did we identify gross evidence of spinal stenosis or of shortening and deformity of bones; however, the above three animals in group 5 (number 43, 51, 56) had obvious narrowing of metaphyseal growth plates and, in some cases, focal closure of metaphyseal growth plates could be seen with the naked eye. Four out of 5 pigs in group 5 had obvious overgrowth and irregularity of cartilage at the costochrondral junction of ribs sectioned at necropsy. Bone measurements (femur and humerus length, vertebral canal diameter and vertebral body length and width) were not significantly different in any of the groups.

**Microscopic Findings**

Significant microscopic lesions in soft tissues were confined to the liver, adrenal gland, skin of the ear, oral mucocutaneous junction and inguinal lymph node. Microscopically, Ito cells could be identified in the liver of all pigs, control and treated. In control pigs, however, Ito cells were few in number, had small lipid vacuoles and were found predominantly in the periportal area of the hepatic lobule. The number of Ito cells in the liver was increased in pigs from groups 2 and 5 (high vitamin A groups); here lipid vacuoles were large and Ito cells were found in all zones of the liver lobule. Increased numbers of hepatic Ito cells appeared to reflect elevated levels of vitamin A in the liver (table 7). While no lesions were identified in the adrenal glands of pigs in the 7 day trial (groups 1 and 2), pigs in group 5 (high vitamin A group) had increased vacuolation of epithelial cells in the zona of fasciculata. In addition, the pigs in group 5 that had the most severe clinical signs had increased numbers of large lipid laden perisinusoidal cells - these were most evident in the zona fasciculata adjacent to the zone glomerulosa.

Three pigs in group 5 had delayed healing at the site of ear tag insertion; these animals had severe dermatitis with surface ulceration at this site. Mucocutaneous junctions of pigs number 39, 43 and 56 (all in group 5) had moderate parakaratotic hyperkeratosis with surface erosion or ulceration. Sections of inguinal lymph node from pigs in group 5 appeared more active and had an increased density of lymphoid follicles when compared to other groups.

All animals in all groups had variably sized focal areas of retention of hypertrophic cartilage in metaphyseal growth plates. This was also seen in articular cartilage. These areas appeared as focal areas of growing cartilage that projected into cancellous bone of the metaphysis or epiphysis and which were often associated with the presence of a large blood vessel. Overgrowth and irregularity of cartilage in the costochondral junction of ribs was observed grossly; histologically, this was seen as extensive and irregular retention of hypertrophic cartilage - apparently due to a failure of mineralization. This lesion was observed in 4 of the 5 animals in group 5 but was not seen in other groups. Eosinophilic streaking of metaphyseal growth plates was a common finding in all groups. This lesion was characterized by narrow vertical eosinophilic streaks in the cartilage of metaphyseal growth plates. Areas of eosinophilia usually were acellular, were often associated with the presence of a blood vessel and focal overgrowth of cartilage. Sometimes there were necrotic cells in adjacent cartilage.

Premature closure of metaphyseal growth plates was the most striking feature of the skeletal examination. It occurred in one animal (#54) in group 2 and in four animals in group 5 (see table 8). Only a few areas of closure were near the margin of growth plates; pressure epiphyses were more commonly affected than were traction epiphyses. Vertebrae, the cranial base, $P_3$, the distal radius and the distal metacarpus were not affected in any case. Some affected metaphyseal growth plates appeared normal aside from a discrete area of closure; in other cases the entire plate appeared abnormally narrow with multiple, sometimes large, areas of premature closure. In some cases bony bridges had formed between the epiphyses and the metaphyses. Mineralized spicules of bone, in the area immediately surrounding blood vessels located in growth cartilage, were observed in all animals in group 5 but were not seen in other groups.

Table 1

Calculated Values and Values Determined by Ration Analysis for Selected Ingredients of Control and High Vitamin A Rations

| | Control Diet | | High Vitamin A Diet | |
|---|---|---|---|---|
| | Calculated Value | Assayed Value | Calculated Value | Assayed Value |
| Moisture % | | 10.8 | | 11.1 |
| Protein % | 18.6 | 23.0 | 18.6 | 21.8 |
| Calcium % | 0.95 | 1.19 | 0.95 | 1.02 |
| Phosphorus % | 0.8 | 0.91 | 0.8 | 0.85 |
| Estimated D.E. (Kcal/kg) | 3,450 | 3,351 | 3,450 | 3,395 |
| Vitamin A (I.U.) | 1,750 | 3,840 | 875,000 | 685,500 |

Table 2

COMPOSITION OF MACRO-PREMIX

| MACRO-PREMIX FORMULATION | | | CONTRIBUTION TO FINAL FEED WHEN ADDED AT 50 KG/TONNE |
|---|---|---|---|
| Mono-dical Phosphate | KG/T | 400 | 20 |
| Salt | KG/T | 60 | 3 |
| Limestone | KG/T | 240 | 12 |
| Lysine-HCL | KG/T | 74 | 3.7 |
| Choline Chloride (60%) | KG/T | 60 | 3 |
| Vitamin A | IU/KG | 35,000 | 1,750 (NRC) |
| Vitamin $D_3$ | IU/KG | 16,500 | 825 |
| Vitamin E | IU/KG | 800 | 40 |
| Menadione | MG/KG | 80 | 4 |
| Thiamine | MG/KG | 20 | 1 |
| Riboflavin | MG/KG | 100 | 5 |
| Niacin | MG/KG | 700 | 35 |
| Vitamin $B_{12}$ | MG/KG | 0.5 | 0.025 |
| Pantothenic Acid | MG/KG | 500 | 25 |
| Biotin | MG/KG | 5 | 0.25 |
| Iron | MG/KG | 1,600 | 80 |
| Zinc | MG/KG | 2,000 | 100 |
| Manganese | MG/KG | 500 | 25 |
| Copper | MG/KG | 120 | 6 |
| Iodine | MG/KG | 10 | 0.5 |
| Selenium | MG/KG | 2 | 0.1 |

Table 3

COMPOSITION OF CONTROL AND HIGH VITAMIN A RATIONS (KG/TONNE)

| | Control Diet | High Vitamin A Diet |
|---|---|---|
| Wheat | 534 | 533 |
| Barley | 100 | 100 |
| Soy Meal (47%) | 200 | 200 |
| Skim Milk | 40 | 40 |
| Whey | 40 | 40 |
| Vegetable Fat | 31 | 31 |
| Macro Premix* | 50 | 50 |
| Bran | 3 | - |
| Tylazone-240** | 2 | 2 |
| Rovimix A-500*** | - | 1.75 |
| Chromic Oxide | - | 2.25 |

* PMT Product #911900 WCVM Pig MPX50 (Prairie Micro-Tech Inc., Regina, Sask.)

** Tylazone-240, tylosin phosphate 0.0044% and furazolidone 0.022% (Elanco, Calgary, Alberta)

*** Rovimix A-500 Feed Grade 500,000 I.U. Vitamin A/Gram in Acetate and Palmitate Form (Hoffmann-La Roche, Etobicoke, Ontario)

Table 4

EXPERIMENTAL DESIGN AND DIETARY LEVELS OF VITAMIN A

| GROUP | NUMBER OF PIGS | FEEDING METHOD | DAYS ON FEED | LEVEL ADDED OF VITAMIN A/KG FEED |
|---|---|---|---|---|
| 1 | 5 | ad lib | 7 | 1xNRC(1750I.U.) |
| 2 | 5 | ad lib | 7 | 500xNRC(875,000 I.U.) |
| 3 | 5 | ad lib | 27 | 1xNRC (1750 I.U.) |
| 4 | 5 | restricted* | 27 | 1xNRC (1750 I.U.) |
| 5 | 5 | ad lib | 27 | 500xNRC (875,000 I.U.) |

* Feed was restricted to match intake of group 5

TABLE 5

LEVELS OF RETINYL PALMITATE, RETINOL AND TOTAL VITAMIN A IN SERUM AND LIVERS OF PIGS FED NORMAL AND HIGH LEVELS OF VITAMIN A OVER A SEVEN AND TWENTY-SEVEN DAY PERIOD

| Pig # | Group # | Day 0 Serum Vitamin A | | | Day 7 Serum Vitamin A | | | Day 27 Serum Vitamin A | | | Liver Vitamin A | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Retinyl Palmitate | Retinol | Total Vitamin A | Retinyl Palmitate | Retinol | Total Vitamin A | Retinyl Palmitate | Retinol | Total Vitamin A | Retinyl Palmitate | Retinol | Total Vitamin A |
| 34 | 1 | 57 | 255 | 312 | 111 | 365 | 476 | | | | 138 | 59 | 197 |
| 38 | 1 | 61 | 315 | 376 | 307 | 301 | 608 | | | | 120 | 38 | 158 |
| 42 | 1 | 69 | 242 | 311 | 143 | 341 | 484 | | | | 130 | 47 | 177 |
| 49 | 1 | 0 | 206 | 206 | 189 | 269 | 458 | | | | 136 | 115 | 251 |
| 53 | 1 | 54 | 285 | 339 | 311 | 349 | 660 | | | | 132 | 57 | 189 |
| 35 | 2 | 47 | 357 | 404 | 1174 | 370 | 1544 | | | | 3099 | 788 | 3887 |
| 40 | 2 | 115 | 261 | 376 | 2054 | 368 | 2422 | | | | 3610 | 889 | 4499 |
| 45 | 2 | 0 | 81 | 81 | 3260 | 296 | 3556 | | | | 2710 | 845 | 3555 |
| 48 | 2 | 7 | 206 | 213 | 3385 | 350 | 3735 | | | | 1988 | 717 | 2704 |
| 54 | 2 | 60 | 272 | 332 | 2759 | 503 | 3262 | | | | 3272 | 688 | 3960 |
| 31 | 3 | 48 | 262 | 310 | | | | 40 | 391 | 431 | 172 | 37 | 209 |
| 33 | 3 | 12 | 254 | 266 | | | | 33 | 548 | 581 | 140 | 22 | 162 |
| 46 | 3 | 114 | 164 | 278 | | | | 38 | 480 | 518 | 145 | 27 | 172 |
| 47 | 3 | 37 | 203 | 240 | | | | 9 | 420 | 429 | 219 | 39 | 258 |
| 52 | 3 | 14 | 307 | 321 | | | | 62 | 614 | 676 | 238 | 36 | 274 |
| 32 | 4 | 25 | 252 | 277 | | | | 43 | 451 | 494 | 113 | 36 | 149 |
| 37 | 4 | 21 | 273 | 294 | | | | 33 | 510 | 543 | 122 | 24 | 146 |
| 44 | 4 | 17 | 242 | 259 | | | | 51 | 533 | 584 | 330 | 61 | 391 |
| 50 | 4 | 17 | 157 | 174 | | | | 34 | 324 | 358 | 164 | 33 | 197 |
| 55 | 4 | 46 | 327 | 373 | | | | 15 | 571 | 586 | 168 | 43 | 211 |
| 41 | 5 | 43 | 223 | 266 | | | | 1528 | 422 | 1950 | 27967 | 1137 | 29104 |
| 39 | 5 | 33 | 187 | 220 | | | | 2119 | 545 | 2664 | 20177 | 934 | 21118 |
| 43 | 5 | 7 | 186 | 193 | | | | 3863 | 467 | 4330 | 15144 | 788 | 15932 |
| 51 | 5 | 3 | 220 | 223 | | | | 834 | 378 | 1212 | 15493 | 758 | 16251 |
| 56 | 5 | 28 | 251 | 279 | | | | 381 | 410 | 791 | 14895 | 1268 | 16163 |

Table 6

LEVELS OF RETINYL PALMITATE, RETINOL AND TOTAL VITAMIN A IN SERUM AND LIVERS OF PIGS FED NORMAL AND HIGH LEVELS OF VITAMIN A OVER A SEVEN AND TWENTY-SEVEN DAY PERIOD

| Group # | Day 0 Serum Vitamin A | | | Day 7 Serum Vitamin A | | | Day 27 Serum Vitamin A | | | Liver Vitamin A | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Retinyl Palmitate | Retinol | Total Vitamin A | Retinyl Palmitate | Retinol | Total Vitamin A | Retinyl Palmitate | Retinol | Total Vitamin A | Retinyl Palmitate | Retinol | Total Vitamin A |
| 1 | 48±5* | 261±19 | 309±41 | $212^{a}$±41 | 325±18 | $537^{a}$±41 | - | - | - | $131^{a}$±3 | $63^{a}$±13 | $194^{a}$±16 |
| 2 | 46±18 | 235±46 | 281±60 | $2526^{b}$±411 | 377±34 | $2904^{b}$±408 | - | - | - | $2936^{b}$±277 | $785^{b}$±38 | $3721^{b}$±296 |
| 3 | 45±19 | 238±25 | 283±15 | - | - | - | $36^{a}$±8 | 491±41 | $527^{a}$±47 | $183^{a}$±20 | $32^{a}$±3 | $2[illegible]^{a}$±22 |
| 4 | 25±5 | 250±28 | 275±32 | - | - | - | $35^{a}$±6 | 478±43 | $513^{a}$±42 | $179^{a}$±39 | $39^{a}$±6 | $219^{a}$±45 |
| 5 | 23±8 | 213±12 | 236±16 | - | - | - | $1745^{b}$±607 | 444±29 | $2189^{b}$±624 | $18,735^{c}$± 2505 | $978^{c}$±99 | $19714^{c}$± 2540 |

* Mean values ± standard deviation

a,b,c Mean values with the same or no superscript are not significantly different ($p>0.05$)

Table 7

NUMBERS OF LIPID-LADEN PERISINUSOIDAL CELLS FOUND IN LIVER AND ADRENAL GLANDS OF PIGS FED NORMAL AND INCREASED LEVELS OF VITAMIN A

| | PIGS ON HIGH VITAMIN A DIET | | | | | | | | | | CONTROL PIGS | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 7 DAY TRIAL | | | | | 27 DAY TRIAL | | | | | 7 DAY TRIAL | 27 DAY TRIAL |
| | ANIMAL NUMBERS | | | | | ANIMAL NUMBERS | | | | | | |
| | 35 | 40 | 45 | 48 | 54 | 41 | 39 | 43 | 51 | 56 | ALL PIGS | ALL PIGS |
| CELL NUMBERS* IN LIVER | 14 | 20 | 17 | 18 | 19 | 40 | 39 | 39 | 26 | 28 | $\leq 3$ | $\leq 5$ |
| CELL NUMBERS IN ADRENAL GLANDS | 0 | 0 | 0 | 0 | 0 | 3 | 3 | 22 | 21 | 21 | 0 | 3 |
| HEPATIC TOTAL VITAMIN A (5g/g dry wt.) | 1544 | 2422 | 3556 | 3735 | 3262 | 27,967 | 20,177 | 15,144 | 15,493 | 14,895 | $\leq 660$ | $\leq 676$ |

* Total numbers of lipid-laden perisinusoidal cells found in 5 microscopic fields (1000 x magnification)

Table 8

DISTRIBUTION OF PREMATURE METAPHYSEAL GROWTH PLATE CLOSURE IN PIGS FED A HIGH VITAMIN A RATION FOR 27 DAYS

| Pig # | Femoral Head | Distal Femur | Humeral Tuberosity | Humeral Head | Distal Humerus | Proximal Tibia | Distal Metacarpus | Proximal Radius | Distal Radius | P3 | P2 | Tuber Ischium | Thoracic Vertebrae | Cranial Base |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 41 | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 39 | - | - | - | - | - | - | - | - | - | - | C | - | - | - |
| 43 | C* | C | - | C | - | C | - | - | - | - | - | - | - | - |
| 51 | C | C | - | C | C | C | - | C | - | - | C | - | - | - |
| 56 | C | C | C | C | C | C | - | C | - | - | - | - | - | - |

C* = premature growth plate closure

## Discussion

Although vitamin A has long been recognized as an essential nutrient, its mode of action (except in regard to vision) is not clearly defined. Likewise, while there is a large body of literature relating to vitamin A toxicity, the mechanism of toxicity and lesions that occur in affected animals are incompletely understood. More experimental work needs to be done with vitamin A toxicity if we are to recognize lesions in affected animals, interpret liver and serum levels of vitamin A and understand the pathogenesis of the disease.

Clinical signs in affected animals were obvious, quite severe and under normal circumstances, would defy clinical diagnosis. It is clear from these studies that vitamin A toxicity (as described under the section on clinical signs) must be considered in the differential diagnosis of lameness in swine. Although bone pain is a feature of vitamin A toxicosis in man, the source of this pain is not clear - it could be associated with retarded growth of metaphyseal growth plates and changes in pressure and/or tension on the periosteum.

Although high levels of vitamin A had no significant effect on weight gain or feed consumption, it is our impression that by the end of the 27 day trial, some animals were starting to become debilitated and, given a longer period of feeding high vitamin A rations, performance parameters would likely have been affected in a negative way. Hypercalcemia has been reported in vitamin A toxicosis by other workers; however, this did not occur in the experiment described in this report.

Oral mucocutaneous lesions and lesions associated with the insertion of ear tags are difficult to interpret. However, these changes occurred only in group 5 and may be related to the experimental treatment. Cheilitis, stomatitis and gingivitis have been reported in vitamin A toxicosis in man; the possible role of excess vitamin A in delaying the repair of epithelial surfaces is speculative.

Patterns of changes in vitamin A levels in serum and liver, in general, are consistent with other observations reported in the literature i.e. plasma retinol levels are only increased in very late stages of vitamin A toxicosis and increased levels of retinyl esters in the plasma may be the only sign of excess vitamin A storage in the liver. Retinyl palmitate is reported to be much less toxic that retinol. The individual variation in the vitamin A levels in pigs examined at 27 days and the lack of correlation (in group 5) between vitamin A levels in serum and liver and the severity of clinical signs is difficult to explain. However, in human medicine, it is reported that individual liver storage is highly variable. Since the development of clinical signs of vitamin A toxicity depends on the storage capacity of the liver, this may explain different individual tolerances.

An evaluation of the skeletal lesions observed in pigs fed the high vitamin A rations provided new information in this area. While reports in the literature describe narrowing and premature closure of growth plates, they do not provide details regarding the distribution of lesions in the skeleton or the microscopic detail of affected areas. Our study shows that, unlike naturally occurring cases seen in Saskatchewan, vertebral growth plates are not affected in vitamin A toxicity of this duration nor is there evidence of spinal stenosis. The observation that pressure epiphyses are more often effected than traction epiphyses may indicate that weight bearing trauma may be an important factor in the evalution of growth plate closure. While not subjected to statistical analysis, it is our impression that areas of growth plate closure arise in areas of focal overgrowth of cartilage or in focal areas of eosinophilic streaking of cartilage. Findings relating to the hypertrophy and apparent hyperplasia of Ito cells in the liver and adrenal glands in group 5 pigs is of considerable interest. These are perisinusoidal fat storing cells which are believed to be of fibroblastic origin; they function as a site of long-term storage for vitamin A. In our study, these cells become more numerous and more obvious as vitamin A accumulates within the liver - in fact, this change can readily be appreciated in Hematoxalin-Eosin stained tissue sections and where seen, should raise the possibility of vitamin A toxicosis. In man, long-term vitamin A toxicosis is accompanied by intra-hepatic collagen deposition and cirrhosis. Under these circumstances it is posulated that Ito cells may be transformed into fibroblasts which produce type III collagen. Liver sections from our current experiment

have no evidence of fibrosis or cirrhosis - likely because of the short duration of the feeding trial. An increase in the number of Ito cells in adrenal gland was especially evident in pigs that had the most severe growth plate closure and the most severe clinical signs. One can theorize that in these pigs, the vitamin A storage capacity of the liver had been exceeded.

## Conclusions

1. Vitamin A toxicosis causes marked locomotory disturbances (stiffness, reluctance to rise, over extension of fetlocks, etc) in weanling swine. Clinically, this lameness would not be readily distinguished from other causes of lameness in swine.

2. Performance, bone length and size of vertebral canal were not decreased by rations containing excess vitamin A fed over a 27 day period. This is in contrast to some naturally occurring skeletal diseases where spinal stenosis is a prominent feature.

3. High levels of vitamin A cause narrowing and premature closure of growth plates of the bones of the limbs but vertebrae are not affected. Cartilage and other sites, such as the trachea and ear are not affected.

4. Growth plate closure in vitamin A toxicosis is often focal and seldom occurs at the margin at the growth plate. Closure occurs more commonly in pressure than in traction epiphyses. Areas of pre-existing overgrowth or degeneration (eosinophilic streaking) may be the initial sites of premature closure.

5. In spite of causing premature closure of growth plates, no deformity of articular surfaces was observed in pigs fed high levels of vitamin A.

6. Hypertrophy and hyperplasia of Ito cells occurs in the liver and adrenal glands of pigs with vitamin A toxicosis. These cells are readily identified under the light microscope and can serve as an indicator of excess storage of vitamin A.

7. In suspected cases of vitamin A toxicosis it is important to measure retinyl palmitate levels in serum since retinol levels undergo very little change.

## Impact on Agriculture

A. Funds from the Agriculture Development Fund were orginally assigned primarily to provide a stipend for a graduate student - Dr. Shelagh Copeland, a pathologist in training. Pathologists are in strong demand and are in short supply in western Canada; they are a valuable resource to agriculture in terms of recognizing, understanding and preventing diseases in our livestock populations.

B. Experiment - Vitamin A Toxicosis in Swine

This experimental trial simulates on-farm conditions whereby an error has been made in ration formulation and excess vitamin A is fed over a period of weeks. The clinical signs themselves as well as the individual variation in the development of clinical signs of toxicity are of interest.

Our experimental work provides for a better understanding of vitamin A toxicosis in swine. Data relating to clinical signs, clinical pathology postmortem findings as well as blood and liver levels of vitamin A have direct application in diagnostic laboratories and to veterinary clinicians. Findings involving growth plates of the skeleton are of particular interest since many bone diseases in livestock are poorly understood and often go undiagnosed in terms of establishing a cause.

We now are in a better position to suspect or identify vitamin A toxicity by the simple microscopic examination of the liver. In addition we now have reference values for control animals and for animals fed high (known) levels of vitamin A.

Finally a report of this nature will make producers aware that nutrients such as vitamin A are potent and potentially harmful thereby avoiding the attitude that in terms of vitamin supplementation - a lot helps a lot!

## List of Papers Prepared and Talks Given

Manuscripts submitted: none

Manuscripts in preparation:

Copeland S, Doige CE and Rousseaux C. A Study of Vitamin A Toxicosis in Growing Swine. (To be submitted to "Canadian Journal of Veterinary Research").

Presentations: none

## Personnel Involved in Project

| | |
|---|---|
| Dr. Shelagh Copeland (Graduate Student, M.Vet.Sc.) | 100% |
| Dr. C.E. Doige | 10% |
| Cathy Caspell (Technician) | 1% |
| Lois Ridgeway (Technician) | 1% |
| Dr. C. Rousseaux | 1% |

## Equipment Items in Excess of $500.00

No equipment has been purchased with these funds.

## Statement of Expenditures

### Project No. 2.4-37

| | |
|---|---|
| Saskatchewan Agriculture Development Fund grant | $18,300.00 |
| Final payment | 5,000.00 |
| **Total Grant** | **$23,300.00** |

**Expenditures:**

| | |
|---|---|
| Salaries and Fringe Benefits: | |
| Graduate Student - Dr. S. Copeland | $21,984.00 |
| Material and Supplies: | |
| (includes charges for photography and histology) | 1,316.00 |
| **Total Expenditures** | **$23,300.00** |
| **Balance (31 March, 1990)** | **$ 0.00** |